Identification of mutations that suppress fructose-asparagine toxicity in *Salmonella fraB* mutants

Undergraduate Research Thesis

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by

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Abstract

Salmonella enterica is a foodborne pathogen that causes gastroenteritis, leading to approximately 19,000 hospitalizations and 380 deaths each year in the United States. Current efforts to develop new drugs aim to disrupt pathways important to the bacteria's fitness during infection. High throughput genetic screening of *Salmonella* mutants that are less fit in mice has identified the *fra* locus, containing the *fraBDAE* operon, which confers the ability to metabolize fructose-asparagine (F-Asn). Disruption of this pathway, specifically of *fraB*, is bacteriostatic in the presence of F-Asn and causes a significant decrease in *Salmonella* fitness in mice. The observed phenotype is hypothesized to be due to an accumulation of the toxic intermediate 6-phosphofructose-aspartate (6-P-F-Asp). This has been supported by genetics and mass spectroscopy. Our goal was to better understand the mechanism of this toxicity, which has not yet been examined. To do so, a library of T-POP transposon insertions was created in a *fraB* mutant background. Mutants were then selected that could grow in minimal media with glucose and F-Asn. Several mutants were isolated and classified to identify gene products that alleviate the toxicity of F-Asn to *fraB* mutants.

Acknowledgments

I would like to thank Dr. Brian Ahmer and Dr. Anice Sabag-Daigle for their support and guidance as advisors on this project. I would also like to thank all members of the Ahmer lab for their continual willingness to assist with this project.

A special thank you to Dr. Brian Ahmer, Dr. Natividad Ruiz and Dr. Richard Dutton for serving as committee members for the defense of this thesis.

Henry Blunk created the *fraB80*::kan mutant strain used in this project, HMB206. Phage lysates were prepared with assistance from Anice Sabag-Daigle. I set up all growth curves, prepared and submitted DNA for sequencing, and performed all other procedures for this project. Anice Sabag-Daigle graphed data from growth curves, and analyzed results from DNA sequencing.

Chapter 1

Introduction

Salmonella enterica is a foodborne pathogen that causes gastroenteritis and diarrhea [1]. Approximately one million infections occur in the United States each year, with 19,000 requiring hospitalization, and 380 resulting in death. Globally, non-typhoidal *Salmonella* is responsible for tens of millions of infections annually. Healthy individuals can overcome a typical infection in 4-7 days, but children, the elderly, and immunocompromised individuals may require treatment [2]. Severe infections that have entered the bloodstream, or infection of immunocompromised individuals, are treated with antibiotics [3]. However, antibiotics are not prescribed to healthy individuals. The overuse of antibiotics is a growing threat to public health, as it leads to the development of resistant bacteria. Each year, there are at least 23,000 deaths directly caused by these resistant bacteria [4].

One approach to developing new treatment options is designing antimicrobials that disrupt genes that are essential to the pathogen's fitness. High throughput genetic screening has been used to identify such genes of *Salmonella* required for fitness in a mouse model. Any of these genes has the potential of being a drug target, but not all of them are appropriate or practical. An ideal drug target would be specific to *Salmonella*, allowing the normal gut microbiota to remain intact. The drug should be stable to allow transport, cost effective, and have limited side effects.

Through this screening, the *fra* locus was discovered as essential for *Salmonella* fitness in mice [5]. Previously uncharacterized, this locus has now been shown to confer the ability to use the carbon source fructose-asparagine (F-Asn). The *fra* locus includes the *fraBDAE* operon, with

an upstream transcription factor encoded by *fraR* [5]. As shown in Figures 1 and 2, the operon is arranged in opposite order of action of the pathway. The *fra* locus is specific to *Salmonella*, and was thus further examined as a potential drug target.

The first step of the pathway is conversion of F-Asn to fructose-aspartate (F-Asp). This occurs in the periplasm via the periplasmic asparaginase, FraE. F-Asp is then transported across the membrane and into the cytoplasm by the transporter FraA. The sugar kinase, FraD, phosphorylates F-Asp, giving the intermediate 6-phosphofructose-aspartate (6-P-F-Asp). Finally, 6-P-F-Asp is cleaved to aspartate and glucose-6-phosphate by FraB. Glucose-6-phosphate can then feed in to central metabolism.

It was initially believed that the ability to utilize F-Asn was important for *Salmonella*'s fitness in the inflamed intestine [5]. It was suggested that this fitness was provided because F-Asn could be fermented, or respired with tetrathionate. However, further studies have shown that the ability to metabolize F-Asn doesn't provide higher fitness. Rather, the interruption of the F-Asn metabolic pathway with a *fraB* mutation leads to the accumulation of a toxic metabolite, significantly decreasing fitness [6]. Figure 3 illustrates this decreased fitness compared to wild-type when a *fraB* mutant is grown in the presence of F-Asn.

This phenotype is hypothesized to be due to the accumulation of the metabolic intermediate 6-P-F-Asp. This has been confirmed by measurement of 6-P-F-Asp in wild-type and *fraB* mutant cells using mass spectroscopy. As expected, 6-P-F-Asp accumulated in the *fraB* mutant cells during growth on F-Asn [6]. Thus, FraB offers a potential drug target for treating *Salmonella*. The *fra* operon also has the advantage of being highly specific, since *Salmonella* is the only organism known to utilize F-Asn.

Mutants lacking other genes in the operon have been created, but they are not inhibited by F-Asn *in vitro*, and do not have decreased fitness in mice, due to the lack of 6-P-F-Asp [6]. Disruption of *fraE* only partially disrupts the ability of *Salmonella* to grow on F-Asn. However, double mutants deficient in *fraE* and *ansB*, a different periplasmic asparaginase, were unable to grow in the presence of F-Asn, suggesting that redundancy makes FraE an inappropriate drug target [6]. This leaves FraB as the most viable drug target in the operon.

The toxicity observed with the accumulation of 6-P-F-Asp may be similar to sugar phosphate stress, which has been found to occur in multiple species. In *Escherichia coli*, cell growth is inhibited when disruption of metabolic pathways leads to the accumulation of galactose-phosphate or glucose-6-phosphate [7,8]. Growth inhibition due to excess glucose-6-phosphate has also been shown in *Salmonella* [7]. In most cases, the mechanism of sugar phosphate toxicity has never been determined, but it has been suggested that the phenotype is due to the depletion of other metabolites and not the accumulation of the intermediate.

A mechanism of resistance was recently described via sugar transport related sRNA (SgrS) [8]. SgrS plays two important roles in allowing resistance. First, SgrS can regulate translation by interacting with mRNA. This interaction can lead to translational repression, often resulting in mRNA degradation, or translational activation. In the case of sugar phosphate stress, SgrS binds mRNA, repressing *ptsG* and *manXYZ* which encode sugar transporters, and activating *yigL*, encoding a phosphatase. SgrS is also translated to give SgrT, which inhibits the PtsG transporter. Acting together, this allows the cell to cope with the accumulating sugar-phosphate by increasing efflux and inhibiting the uptake of additional sugar-phosphate.

The main objective of this project was to identify the mechanism of 6-P-F-Asp toxicity in *Salmonella fraB* mutants. To identify mutations that suppress the toxicity of F-Asn, a mutant

library was constructed using a T-POP transposon in a *fraB* mutant background. Mutants were then selected that have the ability to grow on glucose and F-Asn with tetracycline. The mutations were then transduced back in to a *fraB* background to reduce the possibility of secondary mutations causing the suppressed phenotype. The insertion site of each mutation was sequenced.

Chapter 2

Materials and Methods

Library construction

A mutant library was created by transducing a T-POP transposon into a *fraB* mutant background. To prepare the lysate, *Salmonella typhimurium* TH3923 was grown overnight in LB at 30˚ C. The cells were washed and subcultured to OD_{550} of 0.39. 1 mL of 0.5 mg/mL mitomycin C was added, and allowed to lyse with shaking at 37˚ C for 4 hours. Mitomycin C induces lysogenic bacteria to produce viral particles [9]. The solution was filter sterilized, transferred to a glass tube, and 100μL of chloroform was added.

The transduction was performed immediately after lysate preparation. A 5 mL culture of the *fraB* mutant *fraB*80::kan carrying the altered target specificity transposase plasmid pNK2880 was grown in LB with ampicillin at 37˚ C overnight with shaking. In a sterile flask, 5 mL of the TH3923 lysate was added to 5 mL of the *fraB*80::kan culture, and incubated for 25 minutes at 37˚ C. The cells were washed twice in LB with EGTA, and re-suspended in 50 mL of LB with EGTA for a 60 minute outgrowth. The number of mutants in the culture was determined by titering on LB with tetracycline. Tetracycline was added to the remaining culture, which was allowed to grow at 37˚ C overnight. Two libraries were constructed, the first containing 4,000 mutants, and the second containing 25,000 mutants.

Phenotype Selection

A selection was applied to the library to obtain mutants that could grow in the presence of F-Asn. Eleven culture tubes with 5 mL of minimal media with 5 mM glucose, 5 mM F-Asn, kanamycin, and tetracycline were each inoculated with 100 μL of library and grown at 37 ˚C overnight. Ten cultures were grown from the 25,000 mutant library, and one was grown from the

4,000 mutant library. The overnight selections were titered, and 100 μL of each dilution was plated on LB with tetracycline and EGTA. One colony was selected from each of the 11 cultures, and struck to isolation twice on LB with tetracycline and EGTA. Growth in minimal medium with F-Asn and glucose carbon sources was confirmed. The strains were denoted by BMH2XX series if selected from the 25,000 mutant library and BMH1XX is selected from the 4,000 mutant library.

Mutant Confirmation and Analysis

Each mutant was transduced back into wild type *Salmonella* (14028) and the *fraB80*::kan mutant (HMB206). To do this, P22 phage lysates were made of each mutant. 100 μL of overnight culture in LB was added to ten-fold dilutions of P22 phage. 3 mL of top agar was added to each infection, plated on LB, and incubated at 37 ˚C overnight. 5 mL of LB broth was added to the plate with complete lysis, and shaken overnight at 4 ˚C. The lysate was harvested, filter sterilized, and 100 μL of chloroform was added.

Overnight cultures of 14028 and HMB206 were started in LB and LB with kanamycin, respectively. 100 μL of lysate from each mutant was added to 100 μL of each culture, and incubated at 37 ˚C for 25 minutes. Cells were washed with LB with EGTA twice, and resuspended in 1 mL of LB with EGTA for a 60 minute outgrowth at 37 ˚C. The outgrowth was titered and plated on LB plates with tetracycline, which were incubated at 37 ˚C overnight. Colonies from each transduction were struck to isolation on LB plates with EGTA and tetracycline twice, and then tested for P22 sensitivity on EBU plates. The resulting strains were denoted BMH3XX if transduced into 14028, and BMH4XX if transduced into HMB206.

Growth Assays

Phage sensitive colonies from the BMH4XX series were grown in culture and their phenotype was confirmed in M9 minimal media with glucose and F-Asn at 37 ˚C for 18 hours. OD_{600} readings were taken every hour by a SpecraMax® M5 Multi-Mode Plate Reader.

Since the T-POP transposon contains the tetracycline inducible promoters tet^A and tet^R, all mutants were tested for the growth in the presence of F-Asn and tetracycline. This growth condition was specifically run to examine BMH414, which was unable to grow in the presence of F-Asn following transduction into a *fraB* mutant background.

Cotransduction frequency

To predict and assist with transposon insertion site identification, colonies were patched on LB with kanamycin to calculate the cotransduction frequency of the T-POP mutation with the *fraB80*::kan. The predicted distance of the insertion site from the *fra* operon was calculated using the Wu equation [10].

Genomic DNA Preparation and Sequencing

Genomic DNA from the BMH3XX series was isolated with a GenElute™ Bacterial Genomic DNA kit and sent for sequencing at The Plant and Microbe Genomic Facility at The Ohio State University.

Inverse PCR

To improve the success rate of sequencing results, inverse PCR was performed on isolated genomic DNA from each mutant as described above. The genomic DNA was first digested with the restriction enzyme *Nla*III in 10X CutSmart® Buffer, 10X BSA, and water. The reaction was incubated at 37˚ C for 3 hours, and the enzyme was heat inactivated at 65 ˚C for 20 minutes. A dilute ligation was performed with the digest mixture in 10X ligase buffer and T4 DNA ligase at 15 ˚C overnight. The ligation was purified with a QIAprep® Spin Miniprep Kit.

The DNA was then digested with *DraI* in 10X CutSmart® Buffer, 10X BSA, and water. The reaction was run at 37 ˚C for 3 hours, and the enzyme was heat inactivated at 65 ˚C for 20 minutes. Inverse PCR was run with T-POP specific primers. The product was purified with QIAquick® PCR Purification Kit and submitted for sequencing as above.

Chapter 3

Results

Two libraries containing 4,000 and 25,000 mutants were constructed with the T-POP transposon. Mutants were selected for the ability to grow in minimal media with glucose and F-Asn carbon sources in the presence of tetracycline. Following selection, eleven mutants were chosen for further evaluation. They were rescreened for the ability to grow in the presence of F-Asn. As shown in Figure 4, the ability of all of the mutants to grow on glucose and F-Asn fell in-between the ability of the wild-type and the *fraB* mutant.

Mutations were transduced back into a *fraB* mutant background and rescreened for the ability to grow in the presence of F-Asn and glucose. These mutants were named as BMH4XX series. Mutants transduced back into a 14028 background were named as BMH3XX series. Figure 5 shows that all but one mutant, BMH414, was able to grow.

Growth of BMH414 in minimal medial with glucose and F-Asn carbon sources and the inducer tetracycline was examined. If the transposon inserted in front of a gene, inducing the promoter could alter the mutant's ability to grow in the presence of F-Asn. However, no change was observed, and the strain was discarded. The ten strains able to grow in minimal media with glucose and F-Asn were grown again under the same conditions, but with the addition of tetracycline. No change was observed in their growth (data not shown).

Cotrandsuction frequencies were used to predict the T-POP insertion site distance from the *fra* operon. Table 1 gives the number of kanamycin resistant colonies divided by the total number of colonies patched, the calculated cotransduction frequency, the calculated insertion distance, and the actual insertion distance, as determined by DNA sequencing, when applicable.

Genomic DNA was isolated from each mutant and submitted to sequencing to identify the T-POP insertion site. Seven of the eleven mutants were successfully sequenced. Table 2 gives the interrupted gene and its function for the seven mutants with successful DNA sequencing results. Five were found to be located within the *fra* operon; BMH311, BMH317, BMH319 and BMH330 were in *fraA* and BMH313 was in *fraD*. Two were located outside the operon. BMH310 was in the permease *yhhT*, and BMH312 was in the glycosyltransferase *gtrA*.

Genomic DNA was also used as a template for inverse PCR to increase the success of sequencing results. Thus far, only the insertion site in BMH312 has been successfully amplified using inverse PCR. Sequencing results showed the same insertion site as genomic DNA sequencing.

Discussion

Salmonella enterica is a food-borne pathogen that can cause serious disease in children, the elderly, and immunocompromised patients [1,2]. These patients typically require antibiotics to treat infection, while healthy individuals can overcome it when untreated [3]. The emergence of antibiotic resistant bacteria has necessitated the discovery of novel drug targets to treat infection [4].

High throughput genetic screening identified *Salmonella* genes important for fitness in a mouse model. This screening identified the *fra* locus, which confers the ability to metabolize F-Asn [3]. This locus is specific to *Salmonella*, making it a promising drug target. This specificity would leave commensal gut microbiota intact after treatment, and limit side effects to the patient.

Disruption of the *fraB* gene within the operon leads to a significant decrease in fitness caused by accumulation of the metabolic intermediate 6-P-F-Asp. This decreased fitness could be similar to sugar phosphate stress. *E. coli* has been shown to be inhibited by the accumulation of galactose-phosphate and glucose-6-phosphate [7,8], and *Salmonella* has been shown to be inhibited by the accumulation of glucose-6-phosphate [7].

The mechanism of this toxicity is unknown in most cases, but resistance mechanisms have been described. Sugar transport related sRNA (SgrS) can either regulate translation directly, repressing the sugar transporter genes *ptsG* and *manXYZ*, and activating the phosphatase gene *yigL* [8]. SgrS can also be translated to SgrT, which can inhibit the sugar transporter PtsG. These mechanisms limit accumulation of the intermediate, and prevent the decreased fitness.

To determine genes that encode products that may interact with 6-P-F-Asp, a mutant library was constructed with the T-POP transposon in a *fraB* mutant background. Mutants were selected for the ability to grow in minimal media with glucose and F-Asn, and further examined to identify the interrupted gene.

Eleven mutants with a suppressed toxicity were chosen for further study. Of the eleven, seven have been successfully sequenced. Of these seven, five were within the *fra* operon. The insertion site of BMH311, BMH317, BMH319, and BMH330 was located within the *fraA* gene, encoding the F-Asp transporter FraA. This prevents accumulation of 6-P-F-Asp, since the precursor F-Asp cannot be brought into the cell. The insertion site of BMH313 was located within *fraD*, encoding the sugar kinase FraD. Without the kinase, F-Asp is not phosphorylated, and there is no accumulation of 6-phosphofructose-aspartate.

Following transduction back into the HMB206 background, BMH414 was no longer able to grow in the presence of F-Asn. It was grown again with the addition of tetracycline to see if the insertion was before a gene that could be induced, but it was still unable to grow. For this reason, it was discarded. Possibly, there was a secondary mutation in the original mutant that was lost during the transduction into HMB206 that allowed it to grow initially.

DNA sequencing showed that BMH310 and BMH312 have transposon insertion sites 24,497 and 1,686,944 base pairs from the *fra* operon, respectively. P22 phage packages 48kb of DNA [11]. This means a cotransduction frequency of 11.8% for BMH310, and 0% for BMH312 would be expected, according to the Wu equation [10]. However, the actual frequencies were 60% for BMH310 and 22% for BMH312, suggesting that the insertion was much closer to the operon. We hypothesize that BMH312 has an insertion in an uncharacterized phage gene near the *fra* operon; this would account for the discrepancy between the cotransduction frequency and the DNA sequencing. If the actual insertion site is known, there should be an exact match in the database. But instead, there was only a 92% match between the sequenced genomic DNA of BMH312 and NCBI BLASTn, suggesting that the uncharacterized gene is similar, but not identical, to *gtrA.* It is possible that there is a related phage closer to the *fra* locus that is not present in the published genome sequence.

The success and accuracy of the sequencing will be improved by submitting inverse PCR product instead of genomic DNA. BMH310 had only a short sequence match with the *yhhT* gene, and BMH312 had a high rate of dissimilarity to the *gtrA* gene. This dissimilarity could be due to the fact that the insertion is in an uncharacterized phage gene, as previously discussed, due to differences in laboratory strains, or sequencing error. Inverse PCR sequencing showed the same insertion site as genomic DNA sequencing for BMH312. However, there is still only a 92%

match, suggesting this is not due to sequencing error, but more likely insertion in an uncharacterized phage gene or laboratory strain difference.

The disruption of *gtrA,* a glycosyltransferase, and *yhhT*, a permease, allowed growth of a *fraB* mutant in the presence of F-Asn. This suggests that these genes are important, and perhaps necessary, for the toxicity of F-Asn. Glycosyltransferases, such as *gtrA*, catalyze the biosynthesis of glycans [12]. To do so, a phosphate donor molecule, which can be a sugar phosphate, is transferred to an acceptor molecule. The accumulation of 6-P-F-Asp could lead to it entering biosynthetic pathways, and being incorporated into LPS or peptidoglycan. Incorporation of 6-P-F-Asp instead of the normal substrate could lead to compromised cell wall stability, halting cell growth, and leading to the observed phenotype.

This can be further examined by looking for a changed cell morphology in a *fraB* mutant when grown in minimal media with F-Asn. Though F-Asn is bacteriostatic to the *fraB* mutant, a compromised cell wall could lead to sensitivity to osmotic pressure. Subjecting the cells to osmotic pressure and observing lysis would further support this hypothesis.

When disrupted, *gtrA* is unable to catalyze the reaction, suppressing the toxicity. The permease, *yhhT*, may play a role in relieving toxicity through influx of efflux of an intermediate of the operon, or a small molecule that interacts with a product of the operon. The function of the permease is unique to that of *fraA*, since *fraA* mutants are unable to grow on F-Asn, indicating there is no redundancy of transportation of F-Asp.

Further changes can be made to improve the success of this project. To saturate the selection, a higher number of cultures will be started from the library, with and without tetracycline. 94 mutants will be selected by inoculating a 96-well plate containing minimal media

with F-Asn, glucose, and tetracycline with 2 μL of library. Two wells will serve as controls, with wild-type 14028 and the *fraB* mutant. The outgrowth will be struck to isolation, and a single colony will be chosen from each of the 94 plates. Growth in minimal media with F-Asn and glucose will be confirmed, and the transposon insertion sites will be identified using genomic DNA, or inverse PCR product if genomic DNA sequencing is unsuccessful. Increasing the number of colonies examined will ideally identify more mutations outside of the *fra* operon.

Creation of a larger library is another long-term goal. 25,000 mutants should provide approximately 5.68X coverage of the *Salmonella* genome. This gives a 99.65% chance of each gene getting hit at least once, and a probability of missing a gene of 3.41×10^{-1} [13]. If coverage is increased to 10X, 99.99% of genes are hit at least once, and the probability of missing a gene decreases to 4.53×10^{-5}, giving complete saturation of the genome. However, the multiple transposon insertion sites within the *fra* operon suggest that the library could already be saturated.

Figures

Figure 1. The horizontally acquired *fra* island. The dark genes on the edges are orthologous to *E. coli* genes, while the light colored genes in the middle were presumably horizontally acquired since divergence from *E. coli* (or alternatively deleted from *E. coli* since divergence). *fraR* (*STM14_4332*) is a single gene that encodes a putative transcription regulator. fraBDAE appear to be an operon. *fraB* (*STM14_4331*) encodes a putative F-Asn deglycase. *fraD* (*STM14_4330)* encodes a putative sugar kinase. *fraA* (*STM14_4329)* encodes a putative F-Asp transporter. *fraE* (*STM14_4328*) encodes a putative L-Asparaginase.

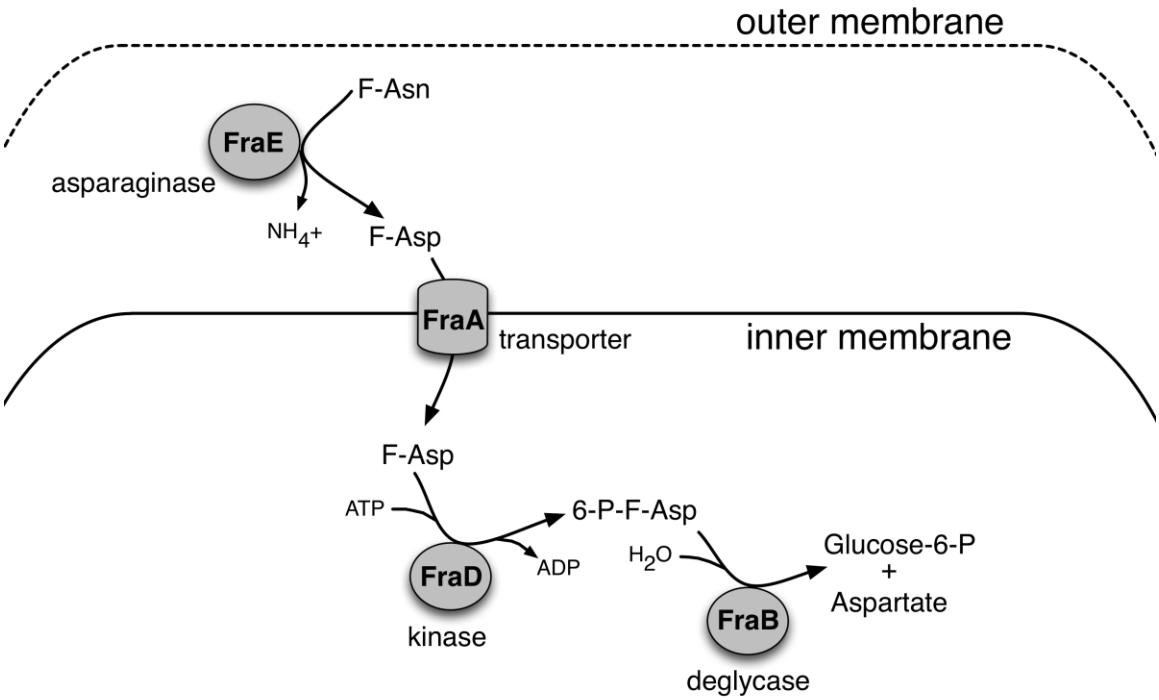

Figure 2. The proposed pathway of F-Asn metabolism. FraE cleaves F-Asn to F-Asp in the periplasm. FraA transports F-Asp into the cytoplasm, where it is phosphorylated to 6-P-F-Asp by FraD. FraB then cleaves 6-P-F-Asp to Glucose-6-Phosphate and Aspartate.

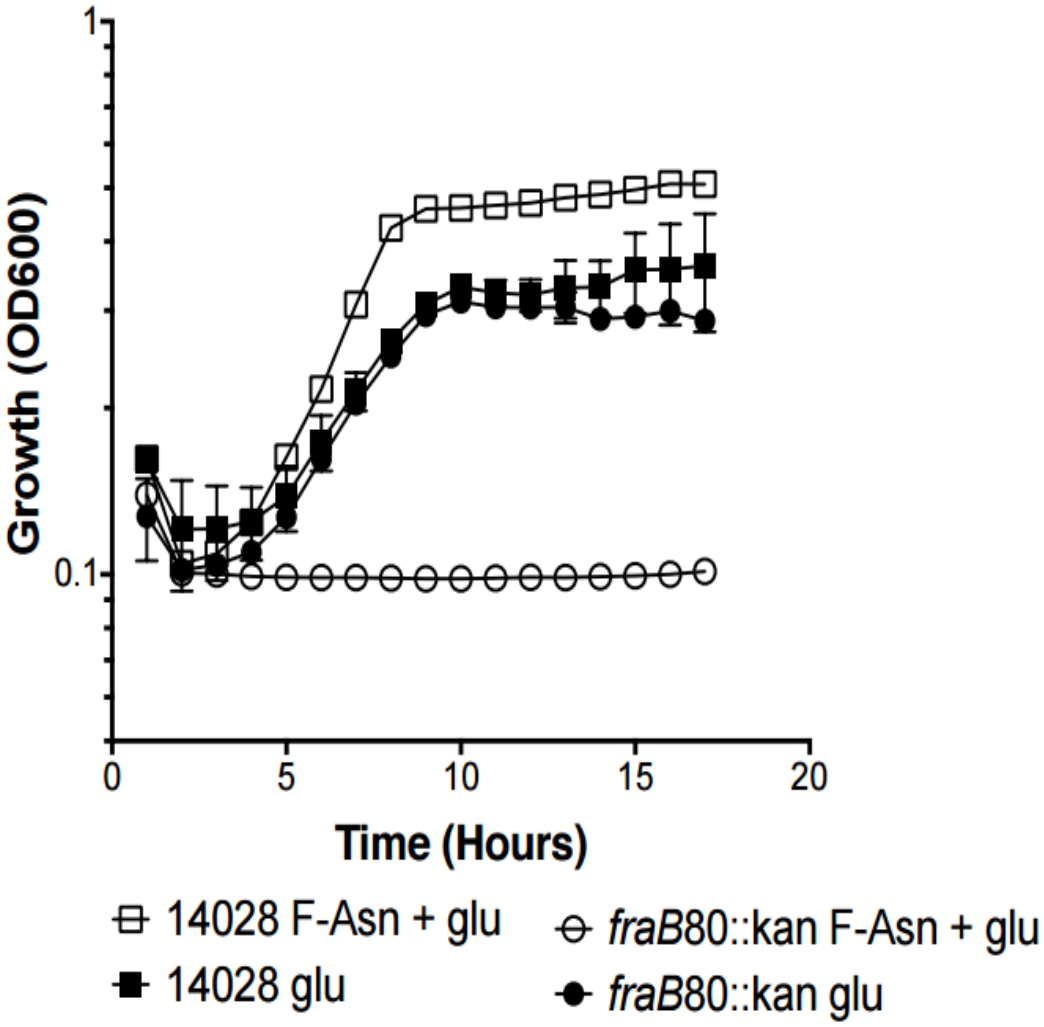

Figure 3. A *fraB* mutant fails to grow in the presence of F-Asn. Wild-type (14028) and *fraB*80::kan (HMB206), were grown in M9 minimal media with either glucose or glucose and F-Asn carbon sources at 37 ˚C for 18 hours. OD_{600} readings were taken every hour by a SpecraMax® M5 Multi-Mode Plate Reader.

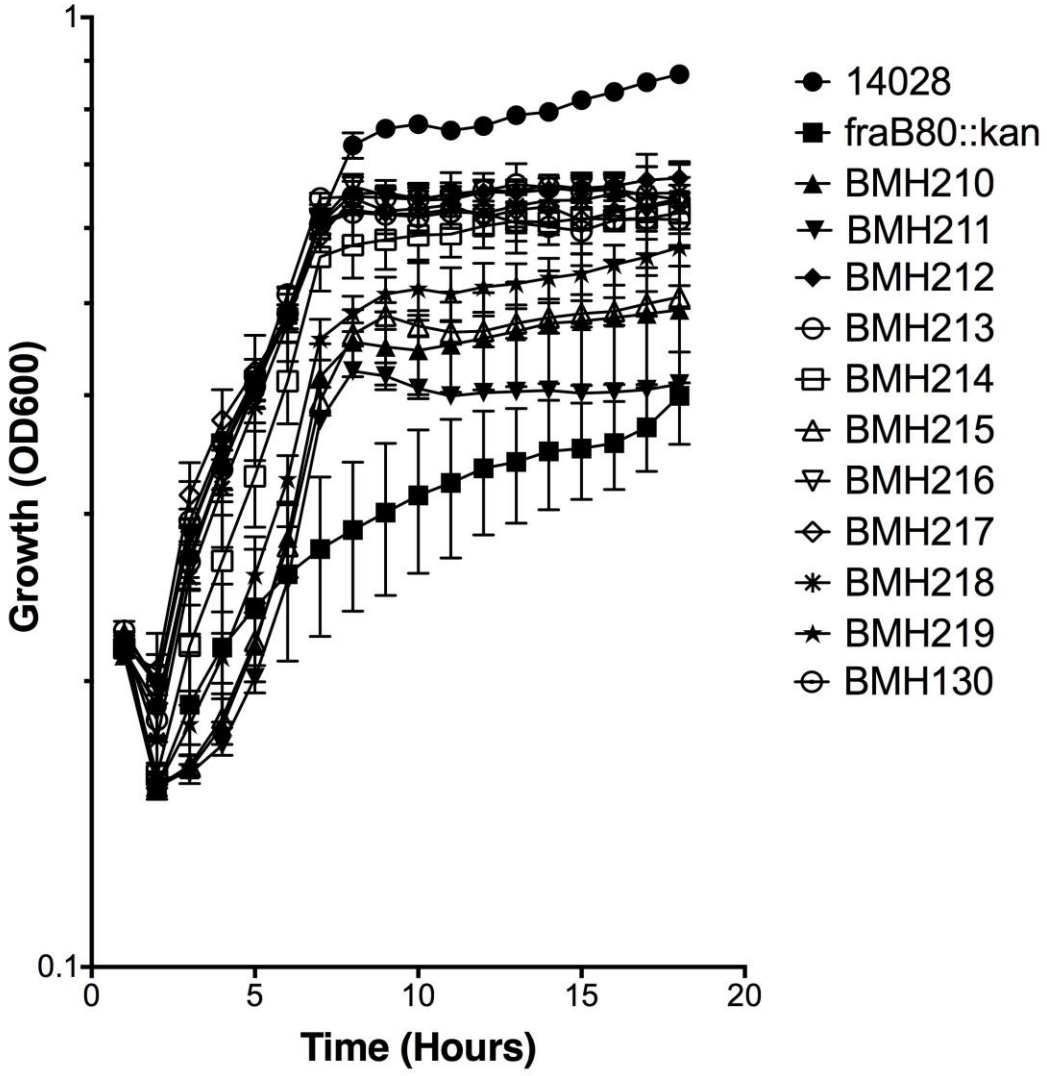

Figure 4. The growth of each mutant selected from the library in M9 minimal media with glucose and F-Asn carbon sources at 37 ˚C for 18 hours. OD_{600} readings were taken every hour by a SpecraMax® M5 Multi-Mode Plate Reader. Growth of all mutants fell in-between wild-type and the *fraB* mutant.

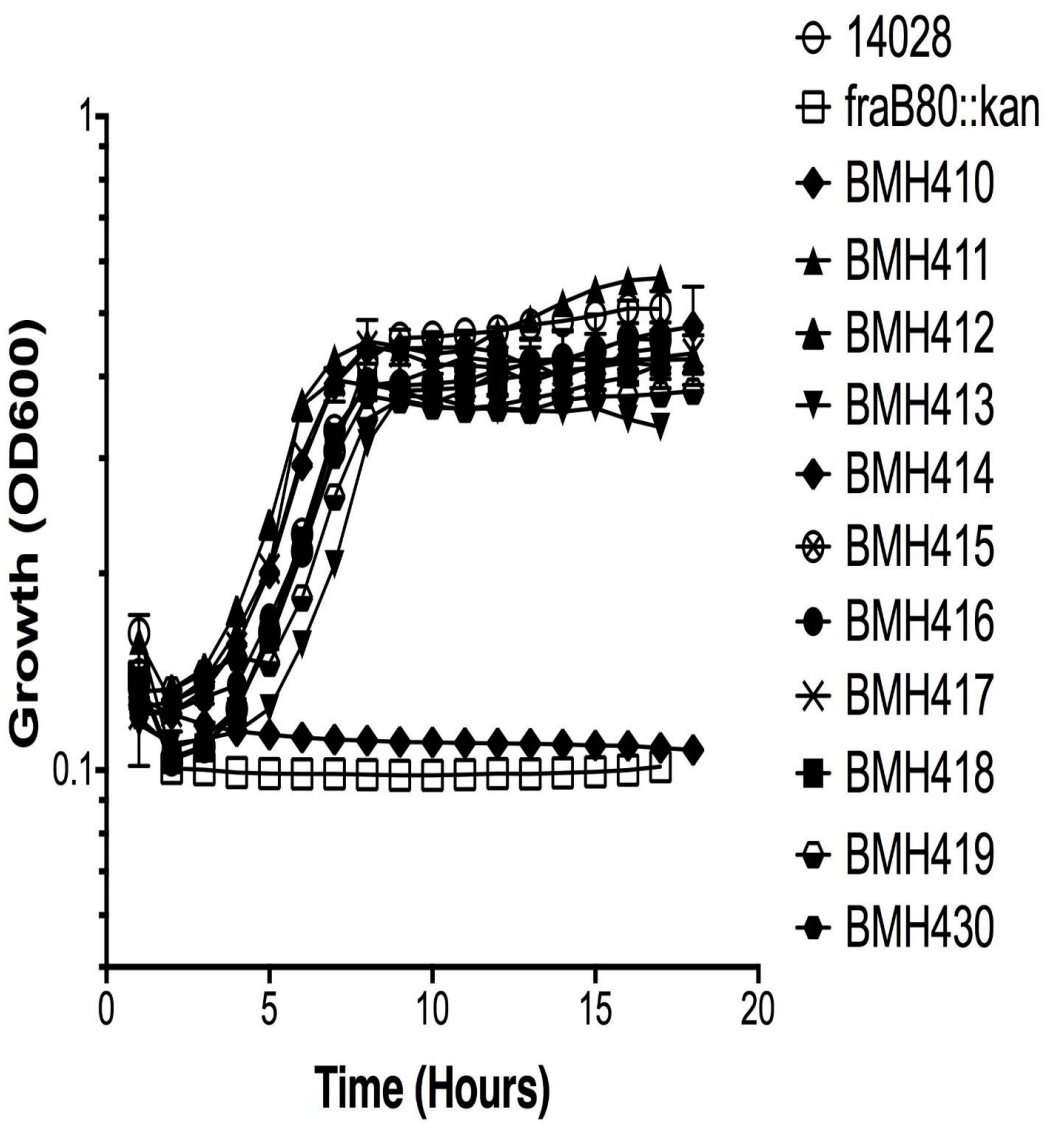

Figure 5. Growth of mutations transduced into a clean fraB mutant background (HMB206). Strains were grown in M9 minimal media with glucose and F-Asn carbon sources at 37 ˚C for 18 hours. OD_{600} readings were taken every hour by a SpecraMax® M5 Multi-Mode Plate Reader.

Tables

Table 1. Co-transduction frequencies and distances from *fraB*.

Mutant	Co-transduction frequency Kanamycin resistant/total patched	Distance from *fraB* based on cotransduction	Distance from *fraB* based on DNA sequence
BMH310	30/50; 60.0%	7,045 bp	24,497 bp
BMH311	3/4; 75.0%	4,114 bp	2,396 bp
BMH312	11/50; 22.0%	17834 bp	1,686,944 bp
BMH313	50/50; 100%	0 bp	1,133 bp
BMH315	33/44; 75%	4,114 bp	Unknown
BMH316	1/8; 12.5%	22,500 bp	2,629 bp
BMH317	35/35; 100%	0 bp	3,058 bp
BMH318	15/20; 75.0%	4,114 bp	Unknown
BMH319	20/20; 100%	0 bp	3,060 bp
BMH330	10/27; 37.0%	12683 bp	3,041 bp

Table 2. Transposon insertion sites identified through sequencing and the function of the disrupted gene.

Mutant	**Transposon Insertion Site**	**Gene Function**
BMH310	STM14_4310; *yhhT*	permease
BMH311	STM14_4329; *fraA*	F-Asp transporter
BMH312	STM14_0651; *gtrA*	glycosyltransferase
BMH313	STM14_4330; *fraD*	F-Asp kinase
BMH317	STM14_4329; *fraA*	F-Asp transporter
BMH319	STM14_4329; *fraA*	F-Asp transporter
BMH330	STM14_4329; *fraA*	F-Asp transporter

Table 3. Strains used.

Strain name	**Genotype**	**Source**
TH4881	LT2 pNK2880(Carb^{R}, Tn*10 tnpA*[ats-1 ats-2])/*fliC5050*::MudJ *fljB5001*::MudCm	14
TH3923	LT2 pJS28(Carb^{r} P22-9^{+})/F′114ts Lac^{+} *zzf-20*::Tn*10*[*tetA*::MudP](Tc^{s}) *zzf-3823*::Tn*10*dTc[del-25](T-POP)/*leuA414 hsdSB* Fels2−	K. Hughes
S. enterica serovar Typhimurim 14028	Wild-type	American Type Culture Collection
HMB206	14028 *fraB*80::kan	6

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